maintains the rejection of the pending claims under § 103(a). Applicants address each rejection under its statutory section below.

Rejection under 35 U.S.C. § 112, second paragraph

In item 2, the Office rejects claims 1, 2, 13-20, 23, and 29 as indefinite for allegedly failing to particularly point out and distinctly claim the subject matter which the Applicants regard as the invention. As the Office addresses several claims individually under this item, Applicants likewise address each claim below.

The Office contends that claim 1 fails to adequately describe the inventive concept. Specifically, there are several different Shiga toxin proteins and the claims should, according to the Office, specify which Shiga toxin protein the humanized monoclonal antibody is binding to. Applicants refer to the instant specification at page 3, lines 7-9 which defines the term "Shiga toxin" as including "Shiga toxin and any other toxins in the Stx1 or Stx2 group or their variants." Thus, in light of the specification, there is no doubt that claim 1 recites humanized monoclonal antibodies that bind to toxins in the Stx1 or Stx2 group or their variants. Applicants therefore respectfully request this rejection be withdrawn.

The Office contends that claim 2 is vague and indefinite because it states that the humanized monoclonal antibody has the "same binding specificity as the antibody selected from the group consisting of . . . ." Applicants assert that the antibodies of claim 2 are not identical to murine antibodies 13C4 and 11E10, as these murine antibodies are not humanized. Rather, the humanized antibodies of claim 2 encompass both the humanized antibodies H13C4 and H11E10 and any other humanized antibody, as defined in claim 1, that has the same binding specificity as any of the four specific antibodies recited in claim 2. Applicants refer to the instant specification

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at page 9, lines 8-10 which defines an antibody with the "same binding specificity" as one with "a level of binding sufficiently detectable in a standard binding assay to distinguish between toxin binding and non-specific background binding as exemplified by appropriate controls." Thus, the recited antibody is not limited to antibodies 13C4, 11E10, H13C4, or H11E10 but includes any humanized monoclonal antibody sharing their binding specificity. Applicants respectfully request withdrawal of the Office's rejection in light of this explanation.

The Office contends that claims 13, 16, and 19 are vague and indefinite because it is unclear what is encompassed by a Shiga toxin type 2 "variant." Applicants assert that one of ordinary skill in the art would understand this common term as indicating a Shiga toxin type 2 protein containing one or more amino acid or nucleotide sequence changes. *See, e.g.*, Rüssmann et al., J. Med. Microbiol., 40:338-343 (1994) (courtesy copy enclosed). In further support, Applicants refer to the instant specification at page 3, lines 2-6, which provides that these variants are "other types of toxins [that] have been discovered and considered to be members of the Stx2 group" and lists three exemplary variants. Applicants therefore respectfully request this rejection be withdrawn in light of this explanation.

The Office contends that claim 14 contains insufficient antecedent basis for the claim limitation "the mouse." Applicants have amended claim 14 to recite ". . . variable region is from a mouse." Applicants submit that this rejection is now moot and should be withdrawn.

The Office contends that claim 16 should be amended to delete the phrase "Figure 6" and the parentheticals, leaving only a reference to the sequence identifier numbers. In addition, the Office notes that the phrase "at least part of" is vague and indefinite. Applicants note that claim 16 does not contain this language. However, claim 15, as established by the Office's suggested claim re-numbering in the Office Action dated February 14, 2000, does contain this language.

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Applicants therefore assume the Office is referring to claim 15 in making this rejection. Applicants have amended claim 15 to recite only the pertinent sequence identifier numbers. Applicants further note that, particularly in light of the dependence of claim 15 on claim 13 which recited binding to Shiga toxin type 2 and variants, that the phrase "at least part of" would be readily understood in the art as referring to a part of the antibody variable region large enough to confer the same or highly similar binding specificity as those variable regions constituted by the amino acid sequences set forth in SEQ ID NOS. 42 and 44. Applicants note that this phrasal explanation also pertains to the Office's rejection of claims 19 and 20. Applicants therefore respectfully request this rejection be withdrawn in light of this explanation.

The Office contends that claim 19 should be amended to delete the phrase "Figure 6" and the parentheticals, leaving only a reference to the sequence identifier numbers. In addition, the Office notes that the phrase "at least part of" is vague and indefinite. Applicants have amended claim 19 to recite only the pertinent sequence identifier numbers and refer to the arguments above regarding parts of sequences. Applicants therefore request this rejection be withdrawn.

The Office contends that claim 20 is vague and confusing because of the term "variant" and the phrase "at least part of." The Office further notes that there is no period at the end of the claim and that the reference to the same complementarity determining regions (CDRs) in two different manners is confusing. Applicants refer to the explanations above regarding toxin variants and parts of the antibody variable region. Moreover, Applicants have amended claim 20 to remove the reference to Figure 6 and add a period at the end of the claim. In light of this amendment, Applicants request that this rejection be withdrawn.

The Office contends that claims 23 and 29 are vague and indefinite due to the terms "fragment" and "derivative." Applicants assert that the term "fragment" refers to a part of the

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antibody variable region or constant region large enough to confer the same or highly similar binding specificity as the humanized monoclonal antibody of claim 1. Applicants further contend that the term "derivative" refers to antibodies with modifications such as deletions, substitutions, or additions to the amino acid sequence that do not appreciably diminish the characteristic binding associated with the exemplified variable regions. *See* the instant specification at page 9, lines 16-18. Thus, claims 23 and 29 generally recite antibodies or smaller peptides that retain the characteristic binding associated with the exemplified variable regions of human antibodies 13C4 and 11E10. Applicants submit that this rejection is no longer warranted in light of this explanation and respectfully request withdrawal of the rejection.

Rejection under 35 U.S.C. § 112, first paragraph

In item 3, the Office rejects claims 1, 2, 13-20, 23, and 29 as containing subject matter which was allegedly not described in the specification in such a way as to enable one skilled in the art to which it pertains to make or use the invention. Specifically, the Office contends that if a single hybridoma has been produced and is intended for a specific function, it is unlikely that the antibody produced will have the required characteristics. The Office further indicates that reproduction of an identical cell line and antibody is an extremely unpredictable event. Applicants respectfully traverse this rejection for the following reasons.

First, as MPEP §2404.02 provides, Applicants may show that a deposit is not necessary even though specific biological materials are required to practice the invention if these biological materials can be made or isolated without undue experimentation. The claimed invention is directed toward humanized monoclonal antibodies based on publically available monoclonal antibodies. As set forth in the instant specification on page 8, lines 7-11, antibodies are divided into a variable region and a constant region. The variable region dictates the antibody's antigen

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specificity, and as its name implies, comes about though a series of genetic recombination events (i.e., VDJ recombination). In essence, the variable region is the most unpredictable segment of an antibody to reproduce. Applicants note, however, that with regard to the claimed humanized 13C4 and 11E10 monoclonal antibodies, the variable regions of these antibodies are known, as recognized by the Examiner in the Section 103 rejection and have been on deposit with the ATCC. Thus, the exact genetic sequences, that give rise to the antigen specificity of the claimed humanized 13C4 and 11E10 monoclonal antibodies, are publicly available. Applicants refer to the instant specification at page 11, lines 5-7 and page 19, lines 13-15, as well as the art cited by the Examiner in the Section 103 rejection, the Spiers paper and the O'Brien patent.

Second, as the instant specification provides on page 8 at lines 13-15 and as recited in claim 1, a humanized antibody's constant region is derived from a human antibody constant region sequence. Applicants also refer to page 13, line 13 and page 22, line 14 which show that a specific human subclass, IgG1, was used to generate the constant region of these humanized antibodies. Applicants submit that the genetic diversity of the constant region, especially within one subclass, is very small compared to the variable region. Applicants further contend that any small variation among amino acid sequences in the IgG1 constant region will not likely give rise to significant differences in functional qualities mediated by the constant region (i.e., complement fixation or opsonization of bacteria). It is for this reason that Applicants also claim derivatives of these antibodies.

Finally, Applicants provide at pages 11-27 of the instant specification extremely detailed instruction on how to recombinantly produce an exemplary antibodies as recited by the claims. These instructions use starting material that is already publicly available (see discussion of ATCC deposits above) and standard IgG1 constant regions sequences and require techniques

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very familiar to one of ordinary skill in the art. These techniques include RNA isolation, PCR mediated cloning (with detailed disclosure of the primers used), and mammalian cell transfection. Given this level of detail in the specification, Applicants contend that one of ordinary skill in the art would be able to make a humanized monoclonal antibody enveloped by the pending claims and to use such an antibody.

In conclusion, given the instant specification's detailed disclosure, Examiner's own acknowledgement of the public availability of the exact variable region sequence used to produce the claimed humanized 13C4 and 11E10 monoclonal antibodies, and the relative lack of variability in the IgG1 constant region, one of ordinary skill in the art could make and use the claimed invention without undue experimentation. Applicants submit that for this reason, any further deposits are unnecessary and respectfully request the Office's rejection of the pending claims be withdrawn.

In item 4, the Office rejects claims 1, 13-20, 23, and 29 as allegedly nonenabled. Specifically, the Office contends that, "While being enabling for a humanized monoclonal antibody which binds to Shiga toxin 1," the instant specification does not contain enablement for humanized monoclonal antibodies that bind to Shiga toxin type 1 variants. Applicants note that none of the rejected claims recite a humanized antibody that binds to a Shiga toxin type 1 variant. Rather, claims 13-20 and claim 29 recite "Shiga toxin type 2 variants." Thus, the Office's rejection is moot and should be withdrawn.

The Office further asserts an alleged lack of enablement for humanized monoclonal antibodies wherein at least part of the variable region is from SEQ ID NOS. 42 and 43. Applicants assume the Office is referring to SEQ. ID NOS. 42 and 44 as recited in claims 15 and 19. The Office also notes that claims 23 and 29 recite fragments or derivatives from a

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humanized monoclonal antibody without providing any functional limitation. According to the Office, these fragments, may not bind to Shiga toxin 1 and the instant specification provides no guidance as to what changes may be made without detrimental effect to the antibody. Applicants traverse this rejection for the following reasons.

First, claims 23 and 29 do provide a functional limitation, as they are either directly or indirectly dependent on claim 1, which recites "[a] humanized monoclonal antibody that binds to Shiga toxin protein. . . ." Thus the functional requirement is that the antibody or fragment or derivative thereof must bind to Shiga toxin protein.

Second, Applicants contend that the basic structure of an antibody is very well known in the art. One of ordinary skill in the art would know where the constant region, variable region, CDR and hinge region are located. Thus, one of ordinary skill in the art would be aware of those regions likely to affect binding to a foreign protein. If, for example, a mutation were made in such a region, and the derivative antibody's ability to bind Shiga toxin protein were in question, Applicants assert that the claimed functionality could be easily determined by using a common ELISA assay as described in the specification at page 26, line 9 to page 27, line 9. One could use this assay to directly detect binding of a modified antibody to Shiga toxin protein. One could also use this assay to test for antibody fragments binding to Shiga toxin by first adding the fragment and checking to see if the fragment blocks binding of a positive control antibody, known to bind Shiga toxin protein. Applicants assert that such assays are routine and do not require undue experimentation. Therefore, Applicants respectfully request the Office withdraw its rejection of claims 1, 13-20, 23, and 29 upon consideration of these explanations.

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Rejection under 35 U.S.C. § 103

In item 5, the Office rejects claims 1, 2, 13-20, 23, and 29 as allegedly unpatentable over Speirs et al. (Can. J. Microbiol., 37:650-653 (1991); Speirs) or O'Brien et al. (U.S. Pat. No. 5,747,272; O'Brien) in view of Shitara et al. (U.S. Pat. No. 5,866,692; Shitara). In response to Applicants' previous arguments, the Office contends that the monoclonal antibodies to Shiga toxin 1 were well known (a point that actually contradicts the Examiner's deposit requirement) and that humanization of a monoclonal antibody already known in the prior art would have been obvious at the time the invention was made since it was a common procedure to allow for passive immunization against human pathogens. The Office also asserts that, because humanizing monoclonal antibodies is a common procedure, it would not have amounted to a motivation to try. Applicants respectfully traverse the Office's rejection.

Applicants contend, arguendo, that just because a technique is common does not necessarily create a motivation to combine it with another technique or reagent. Applicants note that neither Speirs nor O'Brien nor Shitara expressly indicate or suggest a motivation to combine the murine 11E10 monoclonal antibody with a human IgG1 constant region. Even if humanizing monoclonal antibodies is a common technique, this does not suggest that all monoclonal antibodies, whether directed to human pathogens or not, should be humanized. Applicants refer to the abstract of Iwahashi et al. (Mol. Immunol. 36:1079-91 (1999); Iwahashi) which describes a study in which a murine monoclonal antibody with "potential clinical utility" was humanized. These authors found that the murine CDRs themselves can be immunogenic in humans even when grafted onto the framework of human antibody. Of course, the level of immunogenicity will depend on the specific CDRs and thus will likely vary according to the specific antibody. Thus, this example indicates that humanization of antibodies does not necessarily circumvent the

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problem of immunogenicity that humanizing tries to overcome. Applicants also refer to the abstract of Merluzzi et al. (Adv. Clin. Path. 4:77-85 (2000); Merluzzi) which discusses several remaining problems yet to be improved upon before the use of engineered antibodies can be expanded clinically. These problems include improvement in affinity and specificity, demonstration of their safety, and reduction in immunogenicity.

Thus, the Office's contention that a motivation to humanize derives from the common nature of humanizing antibodies is unfounded. Given the above potential caveats to the clinical use of humanized monoclonal antibodies, one of ordinary skill in the art would not necessarily be motivated to combine the variable region of murine monoclonal antibody 11E10 with the constant region of human IgG1 simply by virtue of the familiarity of the technique. Indeed, as the Merluzzi et al. abstract recognizes that "to surmount these problems the molecules will have to be redesigned." As discussed above, none of the three references cited by the Office either alone or in combination, suggest this combination. Applicants therefore respectfully request the rejection of claims 1, 2, 13-20, 23, and 29 be withdrawn.

Conclusion

In view of the foregoing amendments and remarks, Applicants respectfully request the reconsideration and reexamination of this application and the timely allowance of the pending claims.

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Please grant any extensions of time required to enter this response and charge any additional required fees to our deposit account 06-0916.

Respectfully submitted,

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Dated: March 14, 2001

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Appendix to Amendment of March 14, 2001

Amendment to the Claims

14. The humanized monoclonal antibody of claim 13, ~~wherein~~ said non-human variable region is from [the] a mouse.

15. The humanized monoclonal antibody of claim 13, wherein at least part of said variable region is from the sequence set forth in [Figure 6 (]SEQ ID NO:42 and SEQ ID NO:44[)].

18. The humanized monoclonal antibody of claim [16] 17, wherein said human constant region is IgG.

19. A humanized monoclonal antibody which binds Shiga toxin type 2 and Shiga toxin type 2 variants, comprising a constant region and a variable region, wherein:

said constant region is IgG1-kappa, and

said variable region contains at least part of the sequence as set forth in [Figure 6 (]SEQ ID NO:42 and SEQ ID NO:44[)].

20. A humanized monoclonal antibody which binds Shiga toxin type 2 and Shiga toxin type 2 variants, comprising a constant region and a variable region, wherein:

said constant region is IgG1-kappa, and

said variable region contains at least part of the CDR sequences [as set forth in Figure 6, said CDR sequences] located as follows:

Heavy Chain CDRs: CDR1--aa31-35

(SEQ ID NO:44) CDR2-aa50-66

CDR3-aa99-108

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Light Chain CDRs:	CDR1-aa24-40
(SEQ ID NO:42)	CDR2-aa56-62
	CDR3-aa95-103.

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